Expression profile of cathepsins indicates the potential of cathepsins B and D as prognostic factors in breast cancer patients

TAO SUN[1*], DAQING JIANG[2*], LIANG ZHANG[1], QINGLONG SU[3], WANLI MAO[4] and CUI JIANG[1]

Departments of [1]Medical Oncology and [2]Breast Oncology, Liaoning Cancer Hospital and Institute, Shenyang, Liaoning 110000; [3]Department of Medical Oncology, Central Hospital of Chaoyang, Chaoyang, Liaoning 100000; [4]Department of Medical Oncology, Yongchuan People's Hospital, Yongchuan, Chongqing 404000, P.R. China

Abstract. Breast cancer is one of the most prevalent types of cancer in women and contributes to 32% of all female cancer cases. Cathepsins, a family of proteins, are known to have a critical role in human cancers. However, previous studies on the systematic analysis of the role of cathepsin family members in breast cancer are limited. The aim of the present study was to identify biological markers to predict prognosis and treatment response of breast cancer patients, as well as to elucidate novel therapeutic targets. The present study analyzed the expression of six members of cathepsin family, including cathepsins B, G, D, K, L and V in 188 breast cancer tissue specimens using immunohistochemistry. The data showed that all members of the tested cathepsin families featured cytoplasmic staining. Notably, expression of cathepsin L was associated with advanced tumor stages, while cathepsins B and K expression levels were associated with positive estrogen receptor expression; in addition, cathepsin K expression was also demonstrated to be associated with progesterone receptor expression. Cathepsins V and D expression levels were found to be associated with breast cancer metastasis, while the expression levels of cathepsins B and D were associated with poor disease-free survival in breast cancer patients. In addition, univariate analysis demonstrated that breast cancer metastasis to the bone and the expression of cathepsin B protein were associated with poor disease-free survival. In conclusion, the results of the present study indicated that the altered expression of cathepsins, in particular cathepsins B and D, contributed to the progression of breast cancer and poor disease-free survival in breast cancer patients.

Introduction

Breast cancer is the most prominent health problem for women worldwide, affecting one in eight women (1-3). Over the past three decades, there have been numerous advances for breast cancer patients, including earlier detection, increased treatment options and more prevention strategies. However, breast cancer remains to be the second highest cause of cancer-associated mortality in women (1,2). Breast cancer progression, such as metastasis to distant organs, is significantly associated with cancer-associated mortality (1,3). Thus, it is crucial to identify biomarkers to predict prognosis and treatment response, and elucidate novel therapeutic targets of breast cancer.

Proteolytic activity has been reported to be significantly associated with cancer metastasis and altered proteolytic activities was also found to cause a wide range of diseases, including cancer (4). To date, ~500-600 proteases have been identified in the human genome (5). Among them, up to 60 are lysosomal proteases and cathepsins; cathepsins are a group of lysosomal or cysteine proteases, which include 16 members in humans (6-9). Expression of cathepsins has been shown to be upregulated in various types of human cancers (10-12); in addition, altered expression of cathepsins has been associated with tumor angiogenesis, proliferation and invasion (13,14). For example, during cancer progression, translocated or secreted cathepsins were reported to promote tumor cell invasion and metastasis (14).

However, different cathepsins may have different roles in human cancers. Cathepsins B and D were often found to be overexpressed and associated with invasive and metastatic phenotypes of various types of human cancers (15-18). However, the role of each member of the cathepsin family in breast cancer remains to be fully elucidated and to date there have been a limited number of studies on the function of different members of the cathepsin family in breast cancer. A small number of previous studies have reported the altered expression of cathepsins D, B and E in breast carcinogenesis (19-23). In addition, previous studies have reported that cathepsin D was overexpressed in invasive ductal carcinoma

compared with its expression in lobular carcinoma of the mammary glands (21,24,25). Furthermore, overexpression of cathepsin D was associated with lymph node metastasis of breast invasive ductal carcinoma (21).

Notably, the design of small molecular inhibitors targeting cathepsins is a strategy that has been used previously against human cancer, among other diseases (26). Each cathepsin has a different cleavage bond-specificity for substrate proteins, thus allowing for the development of cathepsin-specific inhibitors for targeting different family members (26,27). Such inhibitors include E-64, leupeptin and antipain (26,28,29). These inhibitors are able to selectively suppress cathepsin activity and inhibit tumor growth. For example, anti-cathepsin B and D have been demonstrated to have efficacy in cancer therapy (16,27,30).

Detailed comparison studies of the expression of different members of the cathepsin family in breast cancer are limited. Therefore, the aim of the present study was to analyze the expression of six important members of cathepsin family, including cathepsins B, D, G, K, L and V, in breast cancer tissue specimens. In addition, the expression of these cathepsins was compared with that of reversion-inducing cysteine-rich protein with Kazal motifs (RECK) and vascular endothelial growth factor (VEGF). The expression of these proteins was then analyzed for associations with the clinicopathological data of patients and breast cancer patient survival rates. These data may allow for the development of novel therapies for the treatment of breast cancer patients. In addition, the results of the present study may elucidate cathepsins that have a more dominant role in breast cancer compared with other family members, thus providing evidence for their use in predicting the prognosis of breast cancer patients as well as the development of novel cathepsin-specific treatment options.

Materials and methods

Patient specimens. In the present study, tissue specimens were retrospectively collected from 188 breast cancer patients who underwent treatment at the Liaoning Cancer Hospital and Institute (Liaoning, China). All patients were pathologically diagnosed with breast invasive ductal carcinoma. The present study was approved by the hospital review board of the Liaoning Cancer Hospital and Institute and each patient provided written informed consent for their participation in the study. In addition, follow-up data regarding survival and adverse effects were also collected for the present study at follow-up clinic visits or via phone interview. The detailed clinicopathological data, including age, gender, pathological type, clinical symptoms and stage, lymph node and distant metastasis, expression of estrogen receptor (ER), progesterone receptor (PR) and erythroblastic leukemia viral oncogene homolog 2 (erbB2), menstrual status and laboratory tests, were collected from the patients' medical records.

Immunohistochemistry. All tissue specimens were fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and then dehydrated and embedded in paraffin (Sigma-Aldrich). For immunohistochemistry, the tissue blocks were cut into 4-cm-thick sections and placed in vertical plastic boxes until further use. The sections were dewaxed in analytical reagent

(AR) grade xylene AR and rehydrated through graded alcohol series (AR grade; 100, 95, 90, 80 and 70%). For antigen retrieval, the sections were heated in a citrate buffer (0.01 M; pH 6.0) using a microwave oven (800 W; Haier, Qingdao, China) for 30 min at 93˚C. Following washing three times in phosphate-buffered saline (PBS; Abcam, Cambridge, MA, USA) for 10 min each, the sections were incubated with 3% bovine serum albumin (Abcam) at 37˚C for 30 min. Tissues were then incubated with primary antibodies, including antibodies against cathepsin B (rabbit anti-human polyclonal; 1:50 dilution; cat no. 12216-1-AP), cathepsin K (rabbit anti-human polyclonal; 1:100 dilution; cat no. 11239-1-AP), cathepsin D (mouse anti-human monoclonal; 1:200 dilution; cat no. 2926S), cathepsin G (rabbit anti-human polyclonal; 1:600 dilution; cat no. ab64891), cathepsin L (mouse anti-human monoclonal; 1:100 dilution; cat no. ab6314), cathepsin V (mouse anti-human monoclonal; 1:200 dilution; cat no. ab24508), VEGF (mouse anti-human monoclonal; 1:200 dilution; cat no. ab464154) and RECK (mouse anti-human monoclonal; 1:40 dilution; cat no. 611512) overnight at 4˚C. Cathepsin B and K were obtained from Proteintech (Chicago, IL, USA); cathepsin D, G, L and V, and VEGF were obtained from Abcam; and RECK was obtained from Becton Dickinson (Franklin lakes, New Jersey, USA). The following day, the sections were washed three times with PBS and then further incubated with the corresponding secondary antibodies (Abcam) at 37˚C for 30 min. For color reactions, the sections were washed again with PBS for three times of 5 min each and then stained with a diaminobenzidine solution (Abcam) for up to 5 min. Protein expression in tumor cells was assessed for proportion and intensity score independently by two experienced pathologists. Specifically, the intensity score was obtained by the average intensity of positive cells, which was scored as follows: 0, none; 1, weak; 2, intermediate; and 3, strong. The proportion score was determined according to the proportion of positive cells as follows: 0, none; 1, ≤10%; 2, 11-25%; 3, 26-50%; and 4, >50%. The final score for each protein was calculated by adding the scores for proportion and intensity, then statistically analyzed as low (total score, 0-2) vs. high (total score, 3-8) expression.

Statistical analysis. All statistical analyses were performed using the SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). The Fisher exact test or χ^2 test was used to analyze the difference between two groups, while the hazard ratios and 95% confidence intervals were used to calculate the difference between low and high expression groups. Cumulative survival curves were generated using the Kaplan-Meier method and analyzed using a Cox regression test. Univariate and multivariate analyses were performed to analyze the association between clinicopathological parameters and patient outcomes. $P < 0.05$ was considered to indicate a statistically significant difference between values.

Results

Expression of cathepsins B, D, G, K, L and V as well as RECK and VEGF in breast cancer tissue specimens. In the present study, the expression of cathepsins B, D, G, K, L and V was assessed using immunohistochemistry. In addition, cathepsin expression levels were compared with those of
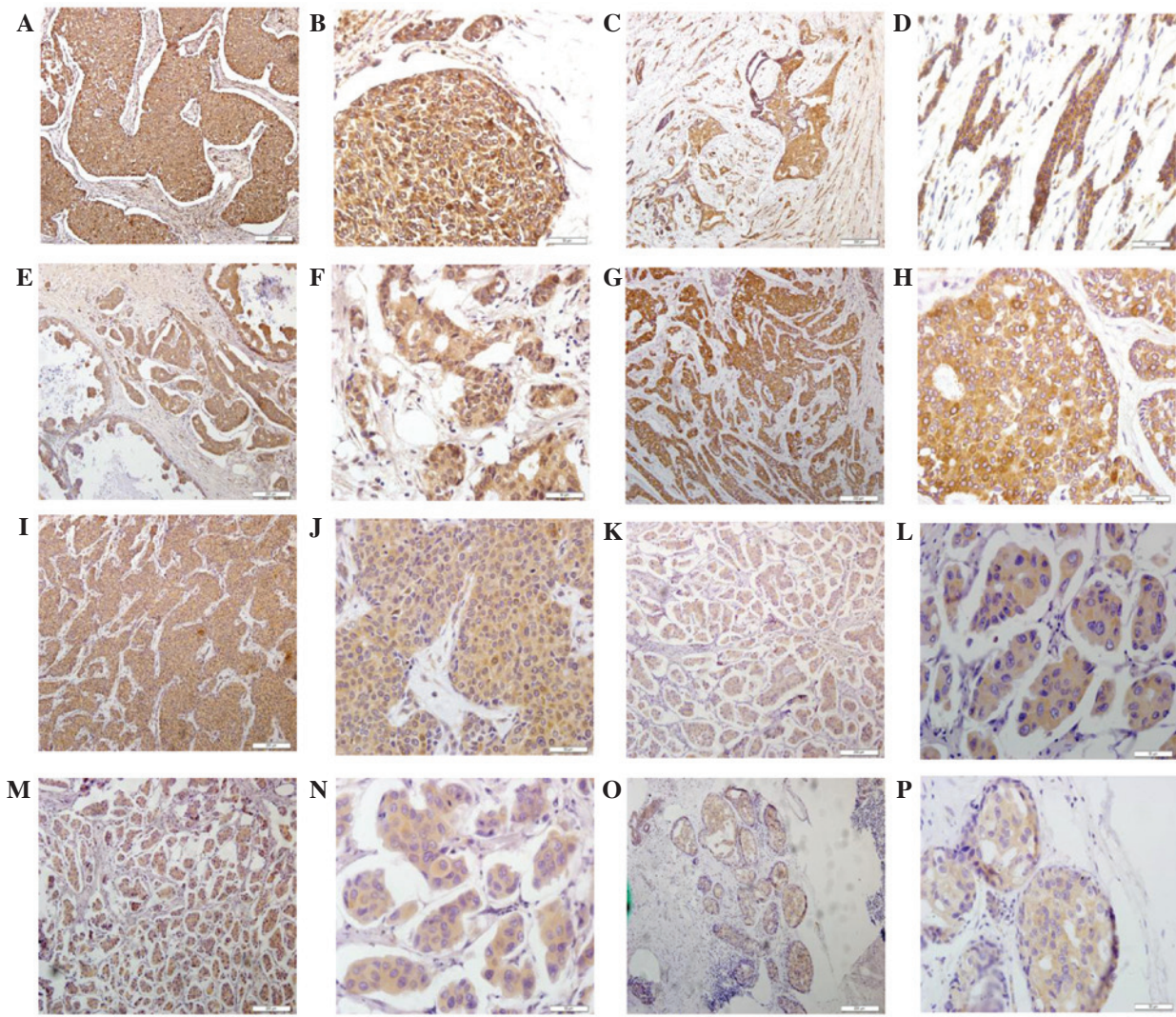
Figure 1. Detection of cathepsins B, D, G, K, L and V as well as RECK and VEGF proteins in breast cancer tissue specimens using immunohistochemistry. Representative images were selected to reflect the expression of (A and B) cathepsin B, (C and D) cathepsin C, (E and F) cathepsin D, (G and H) cathepsin K, (I and J) cathepsin L, (K and L) RECK, (M and N) cathepsin V expression and (O and P) VEGF in breast cancer tissues. Magnification, x200. RECK, reversion-inducing cysteine-rich protein with Kazal motifs; VEGF, vascular endothelial growth factor.

RECK, a metastasis suppressor with protease inhibitor-like domains, and VEGF, which is involved in the proliferation and metastasis of cancer cells. As shown in Fig. 1, all members of these cathepsin families as well as RECK and VEGF demonstrated positive cytoplasmic staining in these breast cancer tissues. Of note, cathepsin B was expressed in 109/142 (76.76%), cathepsin D in 91/155 (58.71%), cathepsin G in 99/171 (57.89%), cathepsin K in 97/143 (67.83%), cathepsin L in 95/166 (57.23%), cathepsin V in 45/164 (27.44%), RECK in 67/175 (38.29%) and VEGF in 100/164 (60.98%) samples of breast cancer tissues. Due to unsuccessful staining in certain samples, staining information could not be obtained from the total 188 samples.

Associations between cathepsins B, D, G, K, L and V as well as RECK and VEGF expression in breast cancer tissue specimens. The expression of certain proteins may have an effect on the expression of other proteins in the same family. In order to determine whether cathepsins interact to effect each others expression, the dependence in expression of different members of the cathepsin family, as well as RECK and VEGF, was analyzed. The results revealed that the expression of cathepsin B was associated with the expression of cathepsins D (P=0.036) and L (P=0.002), while cathepsin D expression was associated with the expression of cathepsins G (P=0.016), L (P=0.003) and V (P=0.006) (Table I). In addition, cathepsin G expression was associated with the expression of cathepsins K (P=0.023), L (P=0.035) and V (P=0.025) (Table I). Furthermore, cathepsin L expression was associated with that of VEGF (P=0.032), while VEGF expression was associated with RECK expression (P=0.001) (Table I).

Association of cathepsins B, D, G, K, L and V as well as RECK and VEGF expression with clinicopathological parameters. The expression of cathepsins B, D, G, K, L and V as well as RECK and VEGF were subsequently evaluated for their associations with the clinicopathological parameters of breast cancer patients. The results demonstrated that cathepsin L expression was associated with advanced tumor

Table I. Association of these protein expressions in breast cancer tissues.

Variable	Cathepsin D			Cathepsin G			Cathepsin K			Cathepsin L			Cathepsin V			VEGF			RECK		
	L	H	P	L	H	P	L	H	P	L	H	P	L	H	P	L	H	P	L	H	P
Cathepsin B																					
-	55	8	0.036	49	17	0.105	36	16	0.830	52	14	0.002	42	18	0.439	30	38	0.100	43	29	0.625
+	41	16		39	25		37	15		33	29		48	15		17	40		35	28	
Cathepsin D																					
-	-	-	-	89	28	0.016	67	26	0.969	82	30	0.003	85	24	0.006	45	68	0.152	80	37	0.248
+	-	-		18	15		21	8		15	18		17	15		8	23		18	14	
Cathepsin G																					
-	-	-	-	-	-	-	75	21	0.023	81	30	0.035	84	25	0.025	41	69	0.925	78	31	0.073
+	-	-		-	-		21	15		25	20		26	18		16	26		22	21	
Cathepsin K																					
-	-	-	-	-	-	-	-	-	-	68	26	0.124	69	23	0.747	36	55	0.180	66	33	0.259
+	-	-		-	-		-	-		21	15		26	10		10	27		22	17	
Cathepsin L																					
-	-	-	-	-	-	-	-	-	-	-	-	-	78	27	0.292	44	58	0.032	72	37	0.111
+	-	-		-	-		-	-		-	-		31	16		12	36		27	24	
Cathepsin V																					
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	62	0.059	70	45	0.673
+	-	-		-	-		-	-		-	-		-	-		11	32		24	18	
VEGF																					
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	47	12	0.001
+	-	-		-	-		-	-		-	-		-	-		-	-		54	46	

L, low expression; H, high expression; VEGF, vascular endothelial growth factor; RECK, reversion-inducing cysteine-rich protein with Kazal motifs.

Table II. Association of cathepsins B, D, C, K, L and V as well as RECK and VEGF expression with clinicopathological parameters from breast cancer patients.

Characteristic	Cathepsin B		Cathepsin D		Cathepsin G		Cathepsin K		Cathepsin L		Cathepsin V		RECK		VEGF	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Age																
<50	41	37	66	21	68	26	59	19	60	31	63	31	62	35	33	59
>50	36	28	56	12	55	21	44	21	55	20	55	15	46	31	29	44
P-value		0.661		0.327		0.997		0.292		0.304		0.103		0.573		0.611
Tumor size																
<2.0 cm	21	14	26	5	25	12	26	7	33	6	24	12	12	26	19	18
2.0-5.0 cm	42	40	76	22	79	27	56	27	66	36	75	24	38	60	71	38
>5.0 cm	14	11	20	6	19	8	21	6	17	9	19	10	12	17	18	11
P-value		0.670		0.733		0.695		0.359		0.075		0.406		0.663		0.298
Histological grade																
Grade 1	18	19	31	10	32	12	26	7	32	13	28	15	33	12	18	27
Grade 2	46	38	71	18	69	29	58	30	66	30	68	25	55	46	33	61
Grade 3	13	8	20	5	22	6	19	3	17	8	22	6	20	8	11	15
P-value		0.615		0.852		0.694		0.099		0.950		0.435		0.051		0.739
Tumor stage																
0/I	11	6	14	1	15	4	14	3	18	0	12	6	7	12	9	9
IIA/IIB	42	41	72	23	77	29	59	23	61	39	69	30	35	64	69	37
III/IV	23	16	33	8	29	13	28	13	32	12	34	10	20	25	27	19
P-value		0.464		0.289		0.723		0.553		0.004		0.582		0.578		0.420
ER																
-	29	14	43	9	44	13	39	8	35	15	42	10	40	17	24	28
+	47	51	78	24	78	34	63	32	79	36	75	36	68	48	37	75
P-value		0.033		0.374		0.300		0.038		0.868		0.081		0.140		0.106
PR																
-	22	17	46	8	45	15	44	9	36	17	43	13	41	18	23	31
+	54	48	75	25	77	32	58	31	78	34	74	33	67	47	38	72
P-value		0.712		0.142		0.545		0.022		0.824		0.304		0.168		0.316
ErbB2																
-	15	21	35	11	37	14	31	13	37	21	34	14	37	12	21	27
+	61	43	86	21	84	33	72	27	76	39	82	32	70	53	40	75
P-value		0.078		0.550		0.920		0.835		0.233		0.888		0.023		0.281

Table II. Continued.

Characteristic	Cathepsin B		Cathepsin D		Cathepsin G		Cathepsin K		Cathepsin L		Cathepsin V		RECK		VEGF	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Lymph node																
Negative	35	25	49	13	58	15	44	14	50	19	46	24	39	35	24	45
Positive	39	39	70	20	63	31	55	26	62	31	70	21	67	29	36	56
P-value		0.330		0.854		0.074		0.307		0.430		0.116		0.023		0.572
Menopausal status																
Pre-menopausal	43	40	67	23	74	27	61	21	63	34	66	33	66	36	33	63
Post-menopausal	33	25	55	10	49	20	42	19	52	17	52	13	41	30	29	39
P-value		0.551		0.127		0.747		0.466		0.152		0.063		0.354		0.282
Distant metastasis																
-	56	39	71	23	79	28	58	25	73	33	69	35	39	68	69	42
+	21	26	51	10	44	19	45	15	42	18	49	11	23	35	39	24
P-value		0.108		0.230		0.574		0.501		0.829		0.035		0.685		0.973
Bone metastasis																
-	62	50	94	27	96	37	75	32	87	44	89	39	50	82	88	50
+	15	15	28	6	27	10	28	8	28	7	29	7	12	21	20	16
P-value		0.601		0.557		0.924		0.374		0.122		0.193		0.872		0.366
Lung metastasis																
-	69	52	99	28	103	39	82	34	97	42	95	41	51	89	91	54
+	8	13	23	5	20	8	21	6	18	9	23	5	11	14	17	12
P-value		0.108		0.624		0.905		0.460		0.748		0.817		0.472		0.675
Hepatic metastasis																
-	71	55	106	31	109	42	88	36	104	45	103	43	57	91	100	56
+	6	10	16	2	14	5	15	4	11	6	15	3	5	12	8	10
P-value		0.154		0.262		0.891		0.471		0.666		0.255		0.463		0.104
Brain metastasis																
-	77	65	120	33	120	47	100	40	113	51	116	46	62	101	109	64
+	1	0	2	0	3	0	3	0	2	0	2	0	0	2	0	2
P-value		0.357		0.459		0.280		0.275		0.343		0.374		0.270		0.069

Table II. Continued.

Characteristic	Cathepsin B Low	Cathepsin B High	Cathepsin D Low	Cathepsin D High	Cathepsin G Low	Cathepsin G High	Cathepsin K Low	Cathepsin K High	Cathepsin L Low	Cathepsin L High	Cathepsin V Low	Cathepsin V High	RECK Low	RECK High	VEGF Low	VEGF High
Chest metastasis																
-	74	58	108	141	111	44	91	37	104	48	107	43	56	94	98	61
+	3	7	14	0	12	3	12	3	11	3	11	3	6	9	10	5
P-value	0.111		0.041		0.488		0.467		0.431		0.564		0.839		0.701	
Opposite breast																
-	77	63	117	32	119	45	99	39	111	49	113	46	60	99	103	65
+	0	2	5	1	4	2	4	1	4	2	5	01	2	4	5	1
P-value	0.121		0.778		0.751		0.686		0.888		0.156		0.827		0.275	

VEGF, vascular endothelial growth factor; RECK, reversion-inducing cysteine-rich protein with Kazal motifs; ER, estrogen receptor; PR, progesterone receptor; ErbB2, erythroblastic leukemia viral oncogene homolog 2.
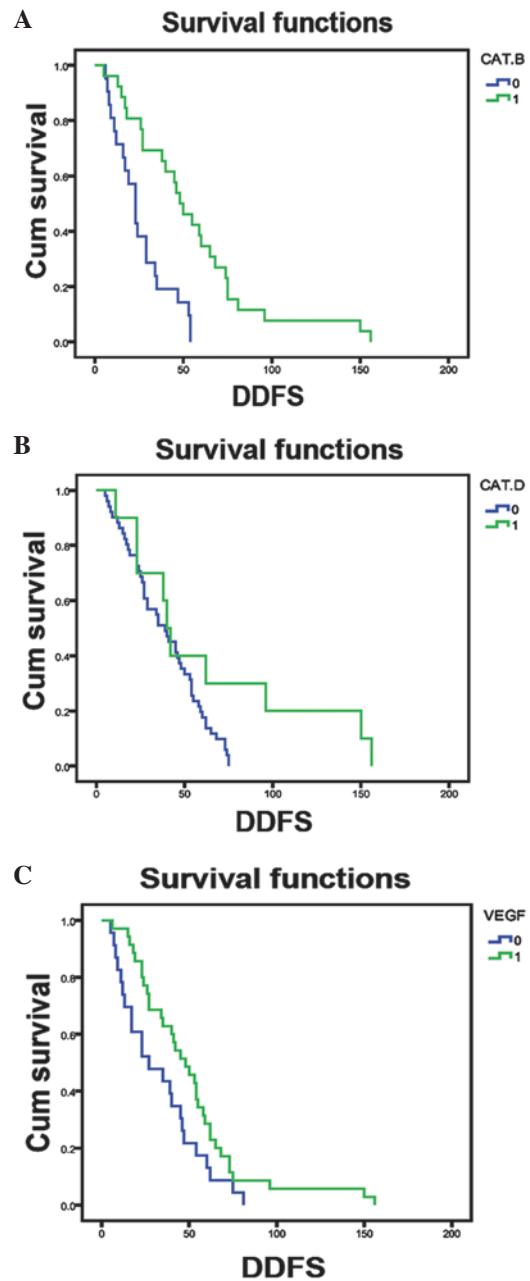
Figure 2. Association of cathepsin B, cathepsin D and VEGF expression in breast cancer tissues with disease-free survival of breast cancer patients. Kaplan-Meier curve of survival of patients according to (A) cathepsin B expression (P<0.001), (B) cathepsin D expression (P=0.050) and (C) VEGF expression (P=0.041). VEGF, vascular endothelial growth factor; Cum survival, cumulative survival; DDFS, distant disease-free survival; Cat., cathepsin; 0, low expression; 1, high expression.

stage, while the expression of cathepsins B (P=0.033) and K (P=0.038) was associated with ER expression (P=0.022) (Table II). In addition, cathepsin K expression was found to be associated with PR expression (P=0.022) and it was revealed that RECK expression was associated with ErbB2 expression (P=0.023) as well as lymph node metastasis (P=0.023) (Table II). Furthermore, cathepsin V expression was associated with distant metastasis (P=0.035), while cathepsin D expression was associated with breast cancer metastasis to the chest (P=0.041) (Table II). However, there was no association identified between protein expression

Table III. Cox regression analysis of disease-free survival according to clinicopathological parameters and protein expression.

	Univariate analysis			Multivariate analysis		
	Exp (B)	95% CI	P-value	Exp (B)	95% CI	P-value
pTNM	1.496	0.939-2.384	0.090	1.652	0.816-3.341	0.163
Bone metastasis	1.780	1.067-2.970	0.027	2.069	0.930-4.606	0.75
Cathepsin B	0.280	0.140-0.561	0.000	0.471	0.216-1.026	0.058
ER	0.625	0.370-1.055	0.078	0.613	0.246-1.529	0.294
PR	0.641	0.384-1.070	0.089	0.470	0.224-0.985	0.046
Cathepsin D	0.461	0.205-1.035	0.061	0.770	0.187-3.165	0.717
VEGF	0.580	0.338-0.995	0.048	0.429	0.209-0.882	0.021

Exp (B), relative risk ratio; CI, confidence interval; pTNM, pathological tumor-node-metastasis; ER, estrogen receptor; PR, progesterone receptor; VEGF, vascular endothelial growth factor.

and patient age, tumor size, histological grade, menopausal status, bone metastasis, lung metastasis, hepatic metastasis, brain metastasis or opposite breast metastasis (Table II).

Association of cathepsins B, D, G, K, L and V as well as RECK and VEGF expression with the prognosis of breast cancer patients. Associations of the expression of cathepsins B, D, G, K, L and V as well as RECK and VEGF proteins with breast cancer patient prognosis were determined. The results revealed that high expression levels of cathepsin D and B and VEGF were associated with poor disease-free survival in breast cancer patients (Fig. 2). However, the expression of other proteins did not show any association with disease-free survival of these patients (data not shown).

Furthermore, univariate analysis data indicated that breast cancer metastasis to the bone and the expression of cathepsin B proteins were associated with poor disease-free survival. In addition, multivariate analysis demonstrated that PR expression was associated with poor disease-free survival and cathepsin B expression was only marginally associated with poor disease-free survival (P=0.058) (Table III).

Discussion

The development of breast cancer involves a progressive multistage process, from premalignant lesion, to ductal carcinoma *in situ*, to invasive carcinoma and then to metastatic disease (19). Given the variability in clinical progression and its high incidence and mortality rates, the identification of biomarkers that are able to predict tumor behavior may be particularly important in breast cancer (19). Therefore, the current study aimed to detected the expression of different cathepsins as well as RECK and VEGF proteins in breast cancer tissue specimens. The results demonstrated that cathepsin B was expressed in 109/142 (76.76%), cathepsin D in 91/155 (58.71%), cathepsin G in 99/171 (57.89%), cathepsin K in 97/143 (67.83%), cathepsin L in 95/166 (57.23%), cathepsin V in 45/164 (27.44%), RECK in 67/175 (38.29%) and VEGF in 100/164 (60.98%) samples of breast cancer tissues. It was also determined that the expression of cathepsin L was associated with advanced tumor stage, the expression of cathepsins B

and K was associated with positive ER expression and that of cathepsin K was associated with PR expression. In addition, the expression of cathepsins V and D was associated with breast cancer metastasis. Furthermore, the expression of cathepsins B and D as well as that of VEGF was associated with poor disease-free survival in breast cancer patients. Univariate analysis revealed that breast cancer metastasis to the bone as well as the expression of cathepsin B and VEGF proteins were associated with poor disease-free survival. In addition, multivariate analysis demonstrated that PR and VEGF expression were significantly associated with poor disease-free survival of the patients, while cathepsin B expression was only marginally associated with poor disease-free survival. Therefore, the present results indicated that targeting cathepsin B and D may have a potential therapeutic benefit for breast cancer patients.

Cathepsins are a superfamily of proteins that are highly expressed in various types of human cancers and have been associated with cancer metastasis (6). Each cathepsin member has relatively different functions and thus, has a different role under normal as well as disease conditions. In cancer, cathepsins have been reported to have various functions, including the regulation of tissue remodeling, cell proliferation and angiogenesis, as well as cancer progression and metastasis (6). Several members of the cathepsin family have previously been studied in breast cancer (31). The results of the present study indicated that the expression of cathepsin V was associated with distant breast cancer metastasis and expression of cathepsin D was associated with breast cancer metastasis to the chest. These results were consistent with those of previously published studies suggesting that members of the cathepsin family were highly expressed in metastatic tumors (10,11,14,19). Cathepsin D protein has been reported to be overexpressed in breast cancer and hyper-secreted by epithelial breast cancer cells (21,32,33). Other previous studies have suggested that cathepsin D expression may be an independent predictor for poor prognosis of breast cancer patients as cathepsin D expression was associated with a high incidence of cancer metastasis (20,34). Consistent with these previous findings, the current study also demonstrated that high expression of cathepsin D protein was associated with poor prognosis of breast cancer patients; however, multivariate analysis failed to

indicate that this was an independent predictor of disease-free survival. In addition, numerous studies have suggested that cathepsin B overexpression was associated with the invasive and metastatic phenotypes of various cancers (15,35). However, the role of cathepsin B in breast cancer remains to be fully elucidated (23,36). In the current study, expression of cathepsin B showed a trend (P=0.058) to independently predict breast cancer disease-free survival; however, this results was not statistically significant. Thus, further studies are required with a larger sample size in order to verify the role of cathepsins in breast cancer and their potential use as biomarkers for disease progression.

Numerous previous efforts have been made to develop specific inhibitors to target the activity of each cathepsin family member (26,37,38). For example, intraperitoneal administration of a highly selective cathepsin B inhibitor, such as CA-074, was reported to reduce the metastatic potential of breast cancer cells in nude mice (39). In addition, inhibitors of the other cysteine cathepsins, including S, L, C and K, are also available (40).

In conclusion, the present study demonstrated that the altered expression of cathepsins, in particular cathepsins B and D, was associated with breast cancer progression and poor disease-free survival. However, future studies are required in order to investigate whether the targeting of these two cathepsins, B and D, may have potential therapeutic use for attenuating the progression of breast cancer.

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